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Title:

Comparison of optomagnetic and AC susceptibility readouts in a magnetic nanoparticle agglutination assay for detection of C-reactive protein

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C-reactive protein (CRP) is an important marker for inflammation. Here, we use magnetic nanoparticles (MNPs) functionalized with CRP antibodies for an agglutination assay to measure the CRP concentration. The presence of CRP results in links between MNPs. In an oscillating magnetic field, clusters of MNPs are not able to rotate as fast as individual MNPs and thus they give a contribution to the measured signal at lower frequencies. Measurements of the response vs. frequency of the applied magnetic field can therefore be used to infer the clustering state of the MNPs and hence the concentration of the target.

Here, we present for the first time a comparison between readouts of the dynamic response of an MNP suspension vs. concentration of a CRP target using an AC susceptometer (magnetic signal) and a recently proposed optomagnetic technique (optical signal) [1].

The non-functionalized beads (black curve in Fig. 1a) only give an optomagnetic signal from single MNPs (negative peak at ~400 Hz). When functionalizing the beads and measuring in CRP free serum (<0.02 µg/ml) the single MNP signal decreases and a signal at lower frequency (~30 Hz) increases (red curve) indicating MNP agglomeration. Increasing the CRP concentration (green and blue curves) the agglomeration increases. However, for the highest CRP concentration (9 µg/ml) aggregation is reduced. This well-known *hook effect* arises when the target saturates the CRP binding sites and prevents agglomeration.

The out-of-phase magnetic susceptibility (fig. 1b) shows the same evolution of the single MNP signal (~300 Hz). Fig. 2 shows that excellent agreement is obtained between the decrease of the peaks heights due to free MNPs in the optomagnetic (V_2') and magnetic susceptibility (χ'') signals.

To conclude, we demonstrate detection of CRP using an agglutination assay and two different physical principles to measure the particle dynamics.

[1] M. Donolato *et al.*, Anal. Chem. **87**, 1622-1629 (2015).

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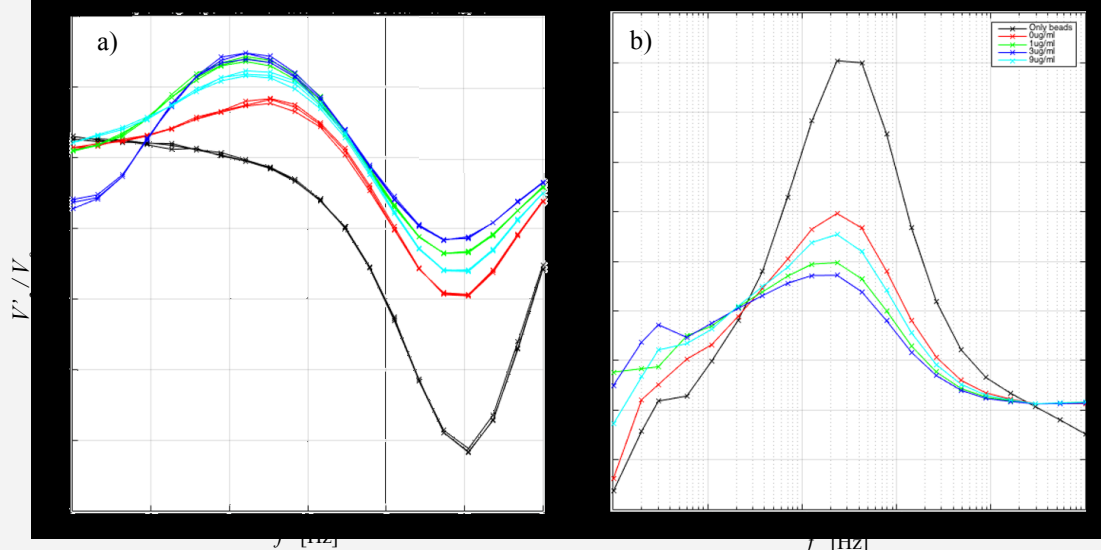


Fig. 1. Data of agglutination assay for non-functionalized beads (black), functionalized beads in the presence of <0.02 $\mu\text{g/ml}$ CRP (red), 1 $\mu\text{g/ml}$ CRP (green), 3 $\mu\text{g/ml}$ CRP (blue), 3 $\mu\text{g/ml}$ CRP (cyan). (a) Real part of the second harmonic optomagnetic signal normalized with the total light intensity. (b) Imaginary part of the AC susceptibility normalized with the infinity frequency limit of the real part, χ'_{∞} .

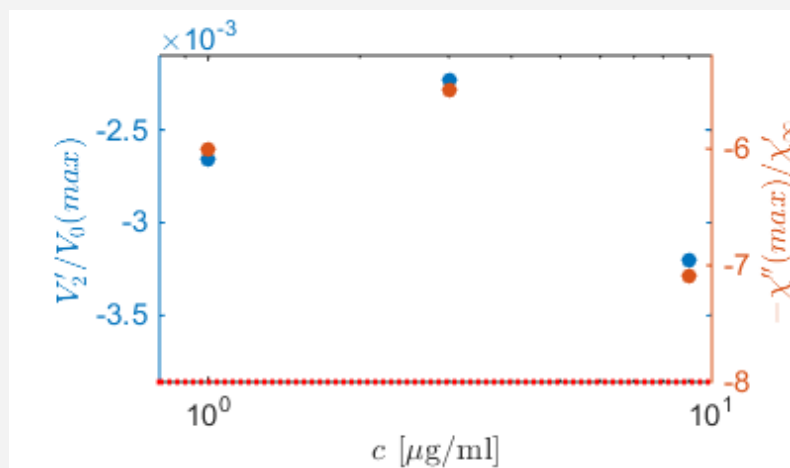


Fig 2. Dose-response curve constructed using the reduction of the single MNP signal at ~300 Hz from optomagnetic data (blue) and AC susceptibility data (red). The dotted line is the value obtained for the CRP-free serum with a CRP concentration <0.02 $\mu\text{g/ml}$.